from coding and non-coding sequences, said sequences being inserted into the body of the vector; and c) a 3' long terminal repeat region comprising a partially deleted U3 region wherein in said partially deleted U3 region [comprises] a polylinker sequence containing a heterologous promoter [not related to the retroviral vector] is inserted, said promoter regulating, after infection of [the] a target cell, expression of [at least] said one [of the] or more sequences selected from coding and non-coding sequences [being inserted into the body of the vector].

32. (Amended) The retroviral vector according to Claim 5, wherein said regulatory element[s and promoters are] is target specific in [their] its expression.

REMARKS

Oath/Declaration

The Examiner states that "[a] new oath or declaration in compliance with 37 C.F.R. 1.67(a) identifying this application by application number and filing date is required" (Office Action, page 3). The Examiner notes that Applicants have claimed priority to PCT/EP95/03445 under 35 U.S.C. §120, and, as such, the declaration must acknowledge the duty to disclose information which occurred between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

Applicants are in the process of obtaining a new executed declaration for filing in the referenced application. Applicants will file the executed declaration with the U.S. Patent Office upon receipt.

Objection of Claim 16

Claim 16 is objected to because "said regulatory elements are" should read "said regulatory element is".

Claim 16 has been amended as suggested by the Examiner, thereby obviating the objection.

Objection of Claim 5 under 37 CFR § 1.75(c)

Claim 5 is objected to under 37 CFR §1.75(c) "as being of improper dependent form for failing to further limit the subject matter of a previous claim" (Office Action, page 4).

Claim 5 has been amended to recite a retroviral vector of Claim 1 which further comprises a regulatory element other than a promoter, thereby obviating the objection.

Rejection of Claims 1, 5, 7, 8-26, 29, 31 and 32 under 35 U.S.C. §112, second paragraph

Claims 1, 5, 7, 8-26, 29, 31 and 32 are rejected under 35 U.S.C. §112, second paragraph "as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention" (Office Action, page 4).

The Examiner states that "Claims 1, 5, 7, 8-26, 28, 29, 31 and 32 "are indefinite for recitation of the phrase 'a heterologous promoter not related to the retroviral vector' because a vector cannot comprise a promoter not related to the same vector since the vector comprises the promoter" (Office Action, pages 4-5). The Examiner suggests deleting the phrase "not related to the retroviral vector" in Claims 1, 17 and 28.

Claims 1, 17 and 28 have been amended as suggested by the Examiner.

The Examiner states that there is insufficient basis for "the target cell" in Claims 1, 5, 7, 8-26, 28, 29, 31 and 32. The Examiner suggests amending Claims 1, 17 and 28 to recite "a target cell".

The claims have been amended as suggested by the Examiner.

The Examiner states that there is insufficient basis for "at least one of the coding sequences being inserted into the body of the vector" in Claims 1, 5, 7, 8-26, 28, 29, 31 and 32. The Examiner suggests amendment Claims 1, 17 and 28 to recite "said one or more sequences selected from coding and non-coding sequences".

The claims have been amended as suggested by the Examiner.

The Examiner states that Claims 1, 5, 7, 8-26, 28, 29, 31 and 32 recite that the 3' end of the vector comprises "a partially deleted U3 region wherein said partially deleted U3 region comprises a heterologous promoter", but it is not clear whether the vector comprises a heterologous promoter or the region that has been deleted comprised a heterologous promoter. The Examiner suggests amending Claims 1, 17 and 18 to clearly recite that the 3' U3 region comprises a heterologous promoter.

The claims have been amended to more clearly indicate that the U3 region comprises a heterologous promoter.

The Examiner states that Claim 7 recites "the regulatory elements and promoters" and Claims 8 and 32 recite "said regulatory elements and promoters", but that there is insufficient

basis for these phrases in the claims. The Examiner suggests amending the claims to recite "said regulatory elements".

Claim 8 has been previously canceled. Claims 7 and 32 have been amended as suggested by the Examiner.

The Examiner states that Claim 10 "is indefinite for recitation of the phrase 'based on a BAG vector' because it is not clear what 'based on' means relative to a vector" (Office Action, page 6). The Examiner suggests amending Claim 10 to recite "derived from a BAG vector".

Claim 10 has been amended as suggested by the Examiner.

The Examiner states that there is insufficient basis for the phrase "said coding sequence" in Claims 11 and 12. The Examiner suggests amending Claim 11 to recite "the coding sequence".

Claim 11 has been amended as suggested by the Examiner.

The Examiner states that Claims 20 and 21 "are indefinite because it is not clear whether the claims read on introduction of a retroviral vector into an animal" (Office Action, page 6). The Examiner suggests amending the Claim 20 to delete the phrase "target human or animal cell populations comprising cells of a human or an animal" and substituting the phrase "a human or an animal".

Claim 20 has been amended as suggested by the Examiner.

Rejection of Claims 1, 5, 8, 9, 11, 12, 15-19, 20-25, 28, 29, 31 and 32 under 35 U.S.C. §102(b)

Claims 1, 5, 8, 9, 11, 12, 15-19, 20-25, 28, 29, 31 and 32 are rejected under 35 U.S.C. §102(b) " as being anticipated by Couture et al." (Office Action, page 7). The Examiner states that Couture *et al.* show retroviral vectors comprising a substitution of a portion of the 3' U3 region with the corresponding region of 5 murine retroviruses; that after packaging, the substituted U3 region appears at the 5' LTR and serves as a promoter for all genes in the body of the vector; that different LTR constructs were preferentially expressed in specific cell types; that U3 regions are bound by cellular factors; that their chimeric LTR promoters are active in a cell type specific manner; that promoter suppression or interference may occur within retroviral vectors containing internal promoters; that retroviral vectors have utility in gene therapy methods; and the use of packaging cells lines PA317 and GP&E86 to package their retroviral vectors. The Examiner concludes that "Couture et al. anticipates the claimed invention.

As amended, Applicants' claimed invention relates to a retroviral vector comprising one or more sequences selected from coding and non-coding sequences which are inserted into the body of the retroviral vector wherein the 3' long terminal repeat region of the vector comprises a *partially deleted* U3 region, and a *polylinker sequence containing a heterologous promoter* is inserted into the partially deleted U3 region, and wherein the promoter regulates, after infection of a target cell, expression of one or more of the sequences selected from coding and non-coding sequences.

As indicated in the previously filed amendments, Couture *et al.* substituted the MoMLV U3 region with the U3 region from five closely related murine retroviruses. In particular, Couture *et al.* replaced the entire U3 fragment with the U3 region from the murine retroviral isolates SL3-3, AKV, Xeno, HaMSV and MPSV (Couture *et al.*, page 668, column 2; page 669, column 2). Couture *et al.* do not teach or suggest a retroviral vector in which the 3' long terminal repeat region comprises a partially deleted U3 region wherein in the partially deleted U3 region a polylinker sequence containing a heterologous promoter is inserted, and after infection of a target cell, the promoter regulates expression of one or more of the coding and non-coding sequences.

Thus, Couture *et al.* do not anticipate Applicants' claimed invention, particularly as amended.

Rejection of Claims 13 and 14 under 35 U.S.C. §102(b)

Claims 13 and 14 are rejected under 35 U.S.C. §102(b) "as being anticipated by Couture et al. in light of Miller et al. and Panganiban et al. '84" (Office Action, page 9). The Examiner states that Couture *et al.* shows a retroviral vector LCSN and a derivative of LCSN, and that "their vectors are derivatives of the vectors of Miller et al." (Office Action, page 9). The Examiner cites Miller *et al.* as showing that "their vectors retain the phi+ packaging sequence, but lack the gag, pol, and env genes of a replication-competent retrovirus" and Panganiban *et al.* as showing that "the 3' end of the pol gene encodes the int locus that is required for integration of the reverse transcribed retroviral genome to form a provirus" (Office Action, page 10).

As amended, Applicants' claimed invention relates to a retroviral vector comprising one or more sequences selected from coding and non-coding sequences which are inserted into the body of the retroviral vector wherein the 3' long terminal repeat region of the vector comprises a *partially deleted* U3 region and a *polylinker sequence containing a heterologous promoter* is inserted into the partially deleted U3 region, and wherein the promoter regulates, after infection

of a target cell, expression of one or more of the sequences selected from coding and non-coding sequences.

As discussed above, Couture *et al.* do not teach or suggest a retroviral vector in which the 3' long terminal repeat region comprises a partially deleted U3 region wherein in the partially deleted U3 region a polylinker sequence containing a heterologous promoter is inserted, and after infection of a target cell, the promoter regulates expression of one or more of the coding and non-coding sequences. Miller *et al.* and Panganiban *et al.* do not provide what is lacking in the Couture *et al.* reference.

Miller *et al.* designed "a set of retroviral vectors which facilitate cDNA transfer and expression" (Miller *et al.*, page 986, column 3), one of which is the LNSX retroviral vector used by Couture *et al.* to generate their vectors. As discussed in the previously filed amendments, Panganiban *et al.* ('84) mutagenized cloned spleen necrosis virus and showed that the 3' end of the *pol* gene of the spleen necrosis virus encodes a polypeptide required for DNA integration through interaction with the *att* site. Neither Miller *et al.* not Panganiban *et al.* teach or even suggest partially deleting the U3 region of a retroviral vector and inserting a polylinker sequence containing a heterologous promoter therein.

Thus, Couture *et al.* in light of Miller *et al.* and Panganiban *et al.* do not anticipate Applicants' claimed invention, particularly as amended.

Rejection of Claim 10 under 35 U.S.C. §103(a)

Claim 10 is rejected under 35 U.S.C. §103(a) "as being unpatentable over Couture et al. in view of Price et al." (Office Action, page 11). The Examiner applies Couture *et al.* as above and cites Price *et al.* as showing "a BAG retroviral vector comprising a beta galactosidase reporter gene, and that the vector can be used to identify cells and progeny of cells infected with the vector" (Office Action, page 12). It is the Examiner's opinion that:

> [i]t would have been obvious to a person of skill in the art at the time the invention was made to modify the vector of Couture et al. by basing the construction on a BAG vector of Price et al. because Price et al shows that a vector with a beta-galactosidase reporter gene may be used to identify cells and progeny of cells infected with the vector (Office Action, page 12).

As amended, Applicants' claimed invention relates to a retroviral vector comprising one or more sequences selected from coding and non-coding sequences which are inserted into the

body of the retroviral vector wherein the 3' long terminal repeat region of the vector comprises a partially deleted U3 region, and a polylinker sequence containing a heterologous promoter is inserted into the partially deleted U3 region, and wherein the promoter regulates, after infection of a target cell, expression of one or more of the sequences selected from coding and non-coding sequences.

As discussed above, Couture *et al.* do not teach or suggest a retroviral vector in which the 3' long terminal repeat region comprises a partially deleted U3 region wherein in the partially deleted U3 region a polylinker sequence containing a heterologous promoter is inserted, and after infection of a target cell, the promoter regulates expression of one or more of the coding and non-coding sequences which are present in the body of the vector. Furthermore, Applicants direct the Examiner's attention to the discussion of Couture *et al.* on pages 9-12 of the Preliminary Amendment mailed to the Patent Office on July 16, 1999.

Briefly, as pointed out in the Preliminary Amendment, based on the general state of the retroviral vector art, after integration into the host cell genome transcription of the genes is expected in the Couture *et al.* constructs, but not in the constructs of the present invention. Couture *et al.* replaced the 5' U3 region with a corresponding region from closely related murine retroviruses. Accordingly, the 5' U3 region of Couture *et al.* is essentially unchanged. Thus, it is not surprising to the person skilled in the art that the 5' U3 regions in which only closely related regions and promoters are exchanged still allow transcriptional read-through of the 5' R region. However, in view of the arguments presented in the Preliminary Amendment, the skilled practitioner would not have considered inserting a heterologous promoter not related to the retroviral vector into a partially deleted U3 region for the purpose of directing expression of a foreign gene inserted into the body of the vector, because in this case the practitioner would have expected that a transcriptional read-through of the 5' R region, and, thus expression of the foreign gene would not occur. Furthermore, in the scientific literature it is reported that genetic rearrangement occurs during reverse transcription, especially when heterologous elements are inserted into the U3 region of the LTR (see Junker, U., *et al., Gene Therapy, 2*:639-646 (1995) which was filed as the Exhibit with the Preliminary Amendment). Such genetic rearrangements are without relevance to the vector of Couture *et al.* because no heterologous elements are inserted into the U3 region. In the vector of Couture *et al.*, the U3 region is replaced by a U3 region of a closely related virus.

Price *et al.* do not provide what is lacking in the Couture *et al.* reference. As discussed in the previously filed Amendment A mailed to the Patent Office on September 16, 1998, Price *et al.* applied a β-gal-transducing vector, BAG, "to the study of neural lineage *in vivo* and in culture" and were able to mark cells in both cases (Price *et al.*, page 158, column 2). In particular, Price *et al.* inserted the β-gal gene, the SV40 early promoter and the Tn5 *neo* gene into the body of the pDOL vector, which is derived from the Moloney murine leukemia virus (Mo-MuLV), and used the vector as a cell-lineage marking system applicable to the vertebrate nervous system

There is clearly no discussion in the Price *et al.* reference regarding the manipulation of the U3 region of the pDOL vector for any purpose. The combined teachings of Couture *et al.* and Price *et al.* do not teach or suggest insertion of a polylinker sequence containing a heterologous promoter into a partially deleted U3 region of a retroviral vector wherein the heterologous promoter directs expression of coding sequences in the body of the vector. Therefore, the combined teachings of the cited references cannot provide the requisite expectation of success in doing so.

Thus, the teachings of the cited references, either alone or in combination, do not render obvious Applicants' claimed invention, particularly as amended.

Rejection of Claims 15, 20, 21 and 26 under 35 U.S.C. §103(a)

Claims 15, 20, 21 and 26 are rejected under 35 U.S.C. §103(a) "as being unpatentable over Couture et al. in view of Longmore et al. and Kay et al." (Office Action, page 13). The Examiner applies Couture *et al.* as above. The Examiner cites Longmore *et al.* as showing that "mice infected with a retroviral vector expressing the erythropoietin receptor had increased platelet counts and splenic megakaryocytes" and Kay *et al.* as showing that "hemophiliac dogs infected with a retroviral vector expressing factor IX shows improved levels of clotting and thromboplastin times for greater that 5 months after treatment" (Office Action, page 13). The Examiner states that:

> [i]t would have been obvious to a person of ordinary skill in the art at the time the invention was made to modify the vector of Couture et al. to express a therapeutic protein because both Kay et al. and Longmore et al. show that retroviral vectors may be used to express therapeutically effective levels of a recombinant protein in an animal. Regarding the limitation in claim 15 to a vector comprising a DNA fragment homologous to a cellular sequence, the erythropoietin receptor gene of

Longmore et al. or the factor IX gene of Kay et al. teach such a sequence in a retroviral vector (Office Action, page 14).

As discussed above, Couture *et al.* do not teach or suggest a retroviral vector in which the 3' long terminal repeat region comprises a partially deleted U3 region wherein in the partially deleted U3 region a polylinker sequence containing a heterologous promoter is inserted, and after infection of a target cell, the promoter regulates expression of one or more of the coding and non-coding sequences which are present in the body of the vector. Longmore *et al.* and Kay *et al.* do not provide what is lacking in the Couture *et al.* reference.

As discussed in the previously filed Amendment A, Longmore *et al.* infected mice with a recombinant spleen focus-forming retrovirus (SFFV) expressing an oncogenic erythropoietin (Epo) receptor (EpoR) and showed a relationship between erythropoiesis and thrombopoiesis at the level of the Epo-EpoR signalling pathway. In addition, Longmore *et al.* teach that the SFV-based vectors "may be excellent vehicles for the introduction of genes into multipotent, hematopoietic progenitors, in vitro" (Longmore *et al.*, abstract). Using an amphotropic retroviral vector that encoded the canine factor IX complementary DNA, Kay *et al.* determined that a method for hepatic gene transfer *in vivo* by the direct infusion of recombinant retroviral vectors into the portal vasculature of a hemophilia B dog model, which results in the persistent expression of exogenous genes, may be feasible for the treatment of hemophilia B patients.

There is no discussion in the Longmore *et al.* or Kay *et al.* references regarding the manipulation of the U3 region of their retroviral vectors for any purpose. The combined teachings of Couture *et al.*, Longmore *et al.* and Kay *et al.* do not teach or suggest insertion of a polylinker sequence containing a heterologous promoter into a partially deleted U3 region of a retroviral vector wherein the heterologous promoter directs expression of coding sequences in the body of the vector. Therefore, the combined teachings of the cited references cannot provide the requisite expectation of success in doing so.

Thus, the teachings of the cited references, either alone or in combination, do not render obvious Applicants' claimed invention, particularly as amended.

Rejection of Claim 7 under 35 U.S.C. §103(a)

Claim 7 is rejected under 35 U.S.C. §103(a) "as being unpatentable over Couture et al. in view of Mee et al." (Office Action, page 14). The Examiner applies Couture *et al.* as above. The Examiner cites Mee *et al.* as teaching a retroviral vector comprising a mouse mammary tumor

virus LTR, that the LTR expressed a gene after induction with dexamethasone and that their vector is a potentially powerful tool for the manipulation of gene expression in a variety of cell types. The Examiner states that:

> [i]t would have been obvious to a person of ordinary skill in the art at the time the invention was made to modify the vector of Couture et al. by insertion of a promoter region in a deleted 3' U3 region of a retroviral vector results in the expression of vector genes under the control of the inserted promoter in a cell type specific manner because Mee et al. show that their LTR promoter may be used to manipulate gene expression in a variety of cells types (Office Action, pages 14-15).

As discussed above, Couture *et al.* do not teach or suggest a retroviral vector in which the 3' long terminal repeat region comprises a partially deleted U3 region wherein in the partially deleted U3 region a polylinker sequence containing a heterologous promoter is inserted, and after infection of a target cell, the promoter regulates expression of one or more of the coding and non-coding sequences which are present in the body of the vector. Mee *et al.* do not provide what is lacking in the Couture *et al.* reference.

As discussed in the previously filed amendments, Mee *et al.* teach the construction and properties of a self-inactivating (SIN) retroviral vector containing a hormonally regulated transcriptional element. In particular, Mee *et al,* disabled the 3' LTR of a retroviral vector and cloned the HRE inducible promoter of the MMTV and the *aph* gene directly between the LTRs of the provirus, *i.e.,* into the body of the vector (Mee *et al.*, pages 289-290). Mee *et al.* do not teach insertion of a heterologous promoter into a partially deleted U3 region of a retroviral vector. The combined teachings of Couture *et al.* and Mee *et al.* do not teach or suggest insertion of a polylinker sequence containing a heterologous promoter into a partially deleted U3 region of a retroviral vector wherein the heterologous promoter directs expression of coding sequences in the body of the vector. Therefore, the combined teachings of the cited references cannot provide the requisite expectation of success in doing so.

Thus, the teachings of the cited references, either alone or in combination, do not render obvious Applicants' claimed invention, particularly as amended.

CONCLUSION

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned at (781) 861-6240.

Respectfully submitted,

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